

Comparative morphological study in the genus *Tirumala* (Lepidoptera, Nymphalidae, Danainae): sexual isolation and PTPs

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Abstract The genus *Tirumala* (Nymphalidae, Lepidoptera) is a group belonging to Danainae and consists of 9 species, 2 from Africa and 7 from the Indo-Australian region (Ackery and Vane-Wright, 1984). This is one of the most difficult genera because its member species resemble each other not only in general appearance but also in morphology of the male genitalia. A comparison was made of the microstructure of pheromone transfer particles (PTPs) in the alar pouch for all species in the genus. Here, we report that some definite differences were detected in the microstructure of PTPs between species. The evaluation of PTPs suggests that, in an area where several species fly together, the morphology of PTPs differs from species to species.

Key words *Tirumala*, Danainae, PTPs, microstructure, SEM, courtship behavior, sexual isolation, taxonomic character.

Introduction

The genus *Tirumala* belongs to the Danainae (Nymphalidae, Lepidoptera) and comprises 9 species, 2 (*T. formosa*, *T. petiverana*) from Africa and 7 (*T. gautama*, *T. choaspes*, *T. limniace*, *T. septentrionis*, *T. ishmoides*, *T. hamata*, *T. euploeomorpha*) from the Indo-Australian Region (Ackery and Vane-Wright, 1984). As in many other Danainae butterflies, the larvae feed on Asclepiadaceae and Apocynaceae which contain noxious ingredients.

Tirumala is one of the most difficult genera due to the resemblance of member species not only in general appearance but also in morphology of the male genitalia. There are only a few studies which examined in detail the morphology of the male and female genitalia of *Tirumala* (Shirôzu, 1960; Kawazoé and Wakabayashi, 1977, etc.). As for grouping of species, Ackery and Vane-Wright (1984) only dichotomized the genus, with Afrotropical *T. formosa* treated as the sister-group of the other eight species, called ‘the *limniace* species-complex’.

A unique morphological character of *Tirumala* is the presence of a sex patch, called the alar pouch, on the hindwing cell Cu_{1b} of males (Fig. 2A–B). Inside the alar pouch, there are scale sockets (Fig. 2A, 1) and disk-shaped sockets (Fig. 2A, 2) on the surface and, from these disk-shaped sockets, characteristic long and narrow scales (called cushion scales) grow. A large number of pheromone transfer particles (PTPs) are produced by fragmentation of the cushion scales after a certain period of time (Boppré and Vane-Wright, 1989). It is believed that PTPs migrate to the tip of the abdominal hairpencils and enable females to recognize and accept their partner by scattering pheromones or by directly sticking to their antennae during courtship behavior. If the chemical constituents of the pheromone and their ratio differ from species to species, it is possible that the morphology of PTPs is also species-specific.

Boppré and Vane-Wright (1989) examined the microstructure of PTPs by SEM, but for *Tirumala petiverana* only. We conducted comparative morphological studies focusing on

the microstructure of PTPs in the alar pouch as well as on the external morphology including appearance (venation, markings and sex patch, etc.) and male and female genitalia, and found definite differences in the microstructure of PTPs among seven species (Hashimoto and Yata, 2007).

In the present study, the microstructure of PTPs was examined in detail in all nine species including two additional species. The morphological differences detected earlier in PTPs were confirmed by the results.

Materials and methods

Forty six individuals were examined representing all the 9 species [and 16 subspecies] of the genus *Tirumala* according to Ackery and Vane-Wright (1984). Males of each species were dissected and examined. Materials used in this study were all dried specimens (Table 1). Specimens of *T. gautama* were borrowed from the Research Institute of Evolutionary Biology, Tokyo, those of *T. formosa* from Kitakyushu Museum of Natural History & Human History and *T. euploeomorpha* from the Natural History Museum, London and the Museum of Nature and Human Activities, Hyogo; and for the other species, dried specimens in the collection of the Graduate School of Social and Cultural Studies, Kyushu University were used.

For observation of PTP microstructure, a part of the pocket-shaped alar organ was taken from the male hindwing, opened so that the inside could be seen; the dissected organs were glued onto a metal stub and gold-coated three times for four minutes each using a JEOL JSM-35 ion sputtering system; the inside of the alar pouch was then examined by using a SEM JEOL JSM-5600LV at 15kV voltage and at magnifications of 800 \times , 1,000 \times , 2,500 \times , 10,000 \times and 15,000 \times .

Table 1. Used materials for observation of PTPs microstructure of male *Tirumala*.

species/subspecies	locality	date of capture	number
<i>T. gautama gautama</i>	Hainan	December 1980	2
<i>T. choaspes choaspes</i>	S. Sulawesi	August 2002–March 2004	5
<i>T. c. kroeseni</i>	Buton	August 2004	1
<i>T. septentrionis septentrionis</i>	Vietnam	June 1997	1
<i>Ditto</i>	Hainan	March 2004	2
<i>Ditto</i>	N. Thailand	July 2005	2
<i>T. ishmoides ishmoides</i>	Buton	?	1
<i>T. hamata leucoptera</i>	Irian Jaya	February 2003	4
<i>T. h. orientalis</i>	Philippines	December 1969, August 1998	4
<i>T. h. paryadres</i>	Moa	January 2003	1
<i>T. h. moderata</i>	Vanuatu	October 1982	1
<i>T. euploeomorpha</i>	Santa Ana	August 1965	1
<i>Ditto</i>	San Cristbal	September 1954	1
<i>T. limniace limniace</i>	Thailand	August 2001	1
<i>Ditto</i>	N. India	June 1992	2
<i>Ditto</i>	Taiwan	August 1952	1
<i>T. l. orestilla</i>	Luzon	December 1969	4
<i>T. l. makassara</i>	S. Sulawesi	August 2003	1
<i>T. l. bentenga</i>	Salayar	January 2007	1
<i>T. petiverana</i>	Kenya	November 2004	3
<i>Ditto</i>	Cameroon	March 2004	1
<i>T. formosa mercedonia</i>	Uganda	July 1980	5
<i>Ditto</i>	Zaire	February 2007	1

Results

When examined by SEM, though slightly different, the shape of PTPs of *T. gautama*, *T. choaspes*, *T. ishmoides*, *T. septentrionis*, *T. hamata* and *T. euploeomorpha* was similar and almost spherical (Fig. 1, A–F). Characteristic wrinkles are seen on the surface in *T. gautama* (Fig. 1, A) and *T. choaspes* (Fig. 1, B). They are peculiarly polyhedral and closely similar in *T. limniace* (Fig. 1, G) and *T. petiverana* (Fig. 1, H). They are globular and covered with many projections in *T. formosa* (Fig. 1, I).

Particles measure 1.2–1.5 μm in diameter in *T. gautama* and *T. choaspes* and 1.5–2.0 μm in *T. ishmoides*, *T. septentrionis*, *T. hamata* and *T. euploeomorpha*. They are a little smaller in *T. limniace* and *T. petiverana*, measuring 1.0–1.5 μm in diameter. They are larger in *T. formosa* than in other species of the genus, measuring more than 2 μm in diameter.

Morphology and size were almost stable within each species when several subspecies were available (multiple specimens from different localities for widely distributed subspecies) are examined.

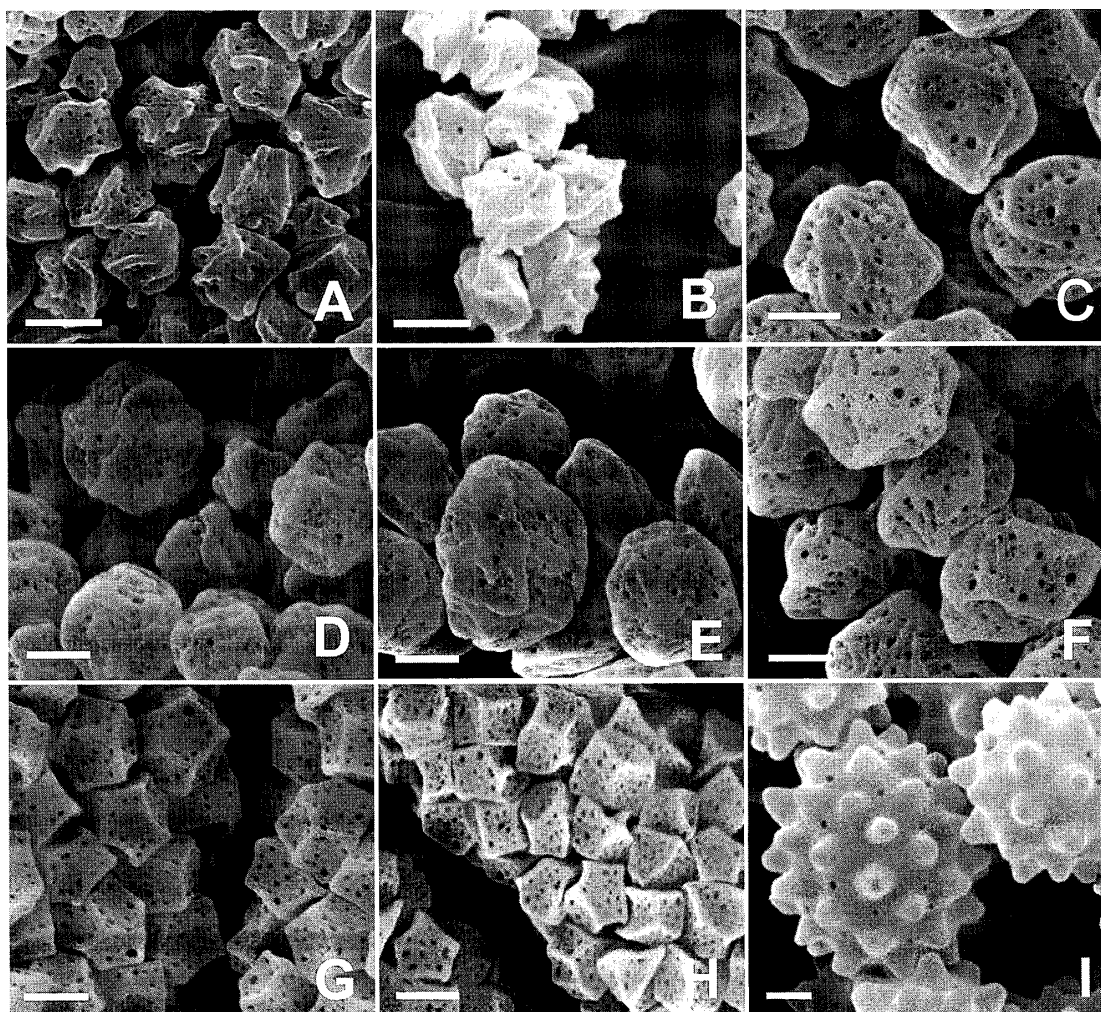


Fig. 1. Microstructure of PTPs of male *Tirumala*. ($\times 15,000$). A. *T. gautama*. B. *T. choaspes*. C. *T. septentrionis*. D. *T. ishmoides*. E. *T. hamata*. F. *T. euploeomorpha*. G. *T. limniace*. H. *T. petiverana*. I. *T. formosa* ($\times 10,000$).

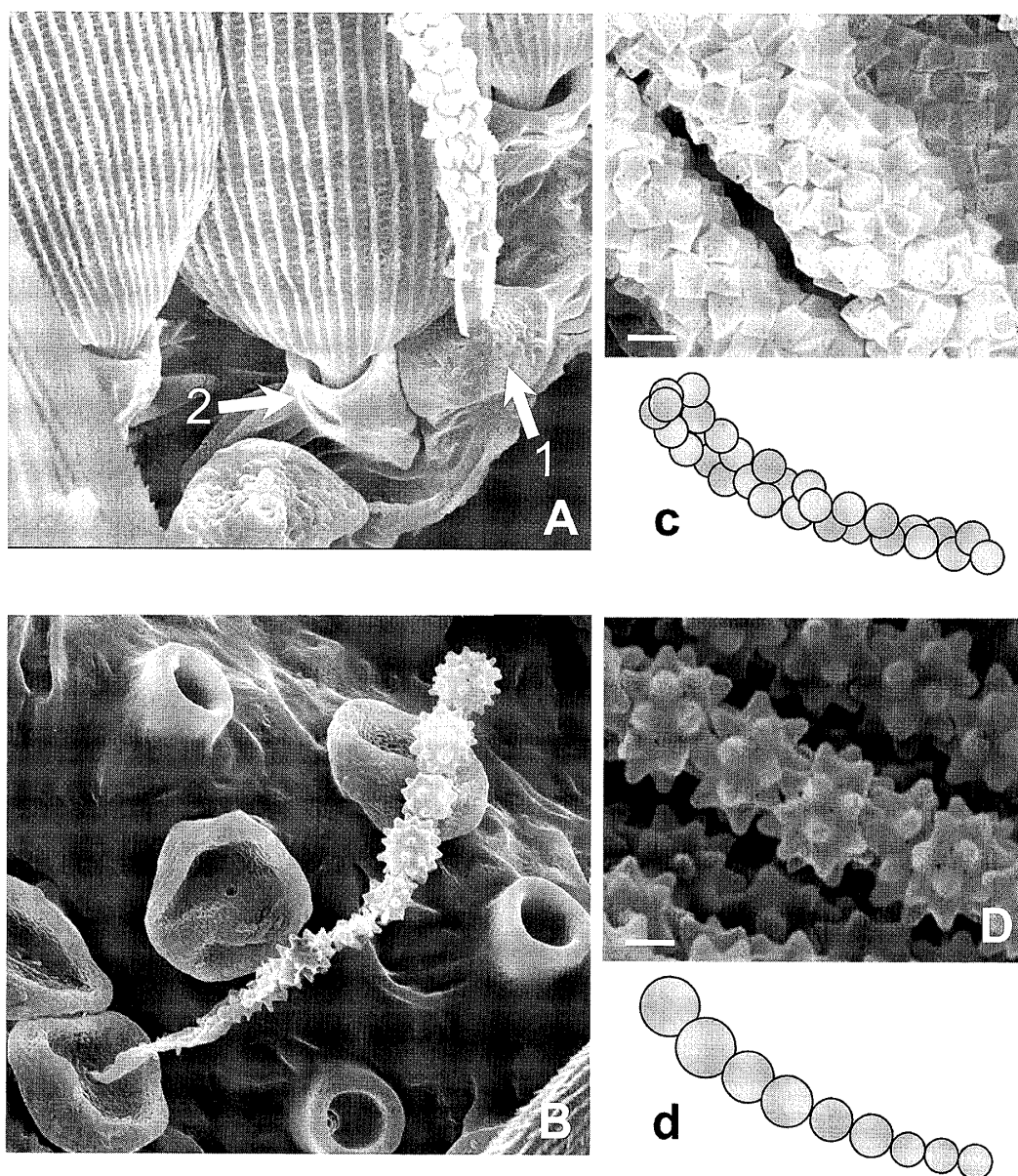


Fig. 2. A-C. Microstructure of PTPs of *Tirumala*. A. Interior of pouch of *Tirumala limniace* showing cushion (1) and pouch (2) scales ($\times 2,500$). B. Interior of pouch of *T. formosa*. C. Cushion scales of *T. petiverana*. D. Cushion scales of *T. formosa*. c-d: Model of cushion scales.

Discussion

The morphology and function of PTPs

The results showed that species with similar PTPs are allopatric. In other words, the evaluation of PTPs suggests that, in areas where several species fly together, the morphology of PTPs differs from species to species (Fig. 3). For *Tirumala* species resembling each other in general appearance, some kind of mechanism for sexual isolation is required. In the genus *Pieris* it is said that the incense material differs among species in ingredients or their ratio (Yata *et al.* 1986). It is probable therefore that the chemical composition of the

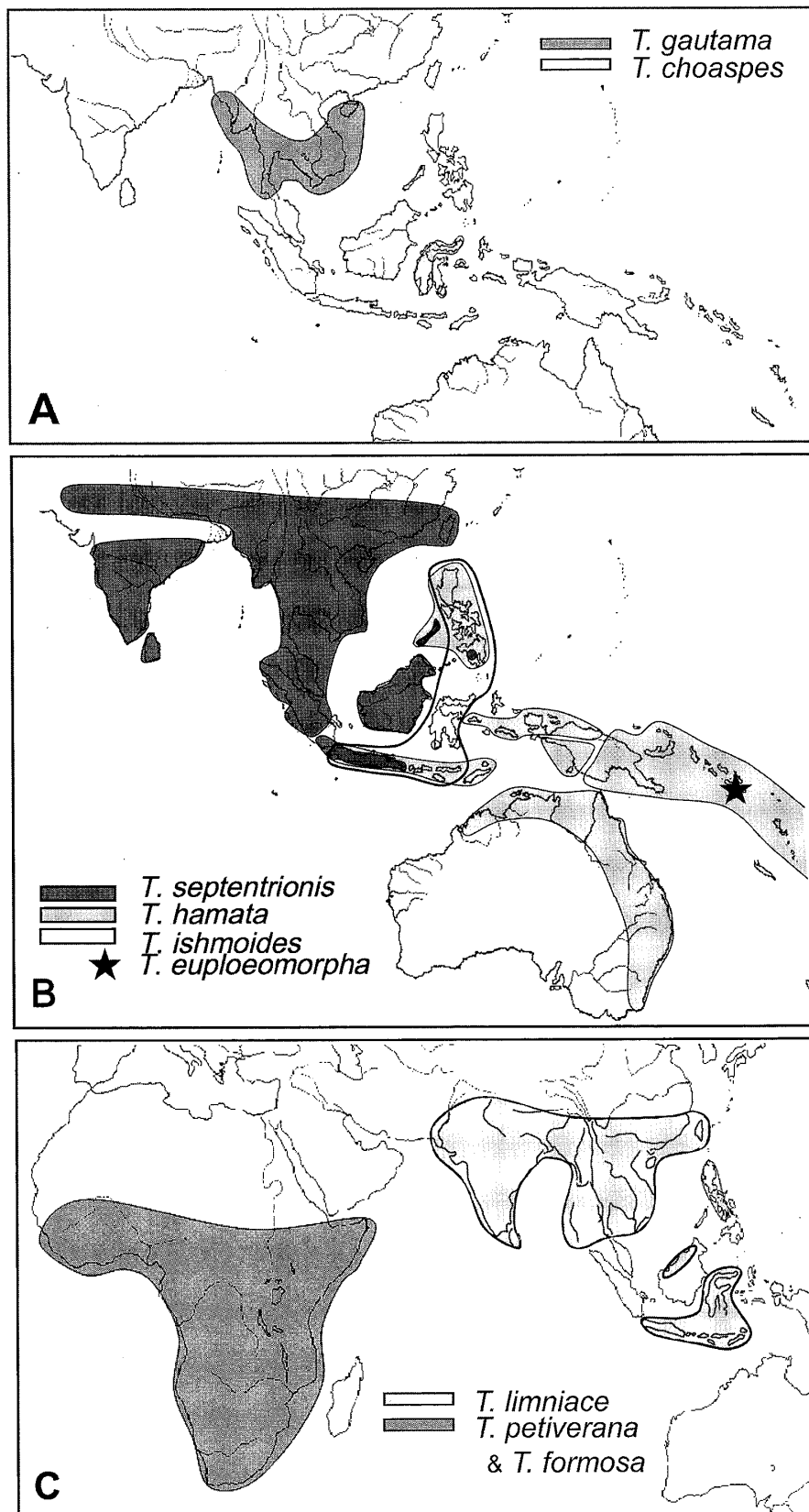


Fig. 3. Distribution maps of some *Tirumala* species. A. *T. gautama* and *T. choaspes*. B. *T. septentrionis*, *T. hamata*, *T. ishmoides* and *T. euploeomorpha*. C: *T. petiverana*, *T. formosa* and *T. limniace*.

pheromone is species-specific and that this specificity is reflected in the morphology of PTPs. (Although Ackery and Vane-Wright (1984) reported PA (pyrrolizidine alkaloids) plants of each species, they did not show their chemical components.)

Four types based on microstructure of PTPs

According to SEM observation, 9 species can be divided into four types based on the morphology and size of PTPs. (1) Characteristic wrinkles, particles measuring 1.2–1.5 μm in diameter (*T. gautama*, *T. choaspes*) (Fig. 1, A–B). (2) Almost spherical, 1.5–2.0 μm in diameter (*T. septentrionis*, *T. ishmoides*, *T. hamata*, *T. euploeomorpha*) (Fig. 1, C–F). (3) Peculiarly polyhedral, 1.0–1.5 μm in diameter (*T. limniace*, *T. petiverana*) (Fig. 1, G–H). (4) Globular and covered with many projections, more than 2 μm in diameter (*T. formosa*) (Fig. 1, I).

The noteworthy point is the resemblance of PTP morphology between *T. limniace* from tropical Asia and *T. petiverana* from Africa in spite of geographical isolation, (Fig. 3, C) suggesting a close relationship between the two (probably vicariant species) as evidenced by the fact that they had been treated as different subspecies of the same species from Talbot (1943) to D'Abrera (1980). Moreover, the morphology of PTPs is also quite similar among four species (*T. septentrionis*, *T. ishmoides*, *T. hamata* and *T. euploeomorpha*). The same applies to the morphology of the male genitalia in these four species (Ackery and Vane-Wright, 1984; Howarth *et al.* 1976, etc.) These resemblances in these morphological characters which tend to be species specific suggest a close relationship among them.

On the other hand, the PTPs of *T. formosa* from Africa are globular and covered with many projections, which is quite different from the other species. Moreover, they are two or more times as large as those of other species. This size is associated with the method of division of the cushion scale (Fig. 2, C–D, c–d.). The cushion scales are divided gradually with time, generating a large number of PTPs (Boppré and Vane-Wright, 1989). The cushion scale is constricted like a rosary in *T. formosa*, while it is divided minutely into a tassel towards the tip in the remaining 8 species. *T. formosa* is thus peculiar not only in the appearance and morphology of the male genitalia but also in its PTPs.

As mentioned above, the present study showed the species specificity of the PTP morphology and its usefulness as a taxonomic character. The classification of the genus *Tirumala* has remained difficult due to the scantiness of differences among species. The microstructure of the PTPs will ensure the identification of species and evaluation of characters difficult so far. In Japan, four *Tirumala* species have been recorded as 'migrant butterflies' (Shirôzu, 2005). So the microstructure of PTPs seems to be useful for the identification of close species, especially *T. limniace* and *T. hamata*.

These results are interesting when considering the speciation process of the genus. Further taxonomic study of the whole genus based on the morphological findings obtained this time and taking into consideration the biogeographic information would clarify the phylogenetic relationships among species.

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摘 要

コモンマダラ属 *Tirumala* における比較形態学的研究: 生殖隔離とフェロモン運搬粒子 PTPs (橋本 恵・矢田 脩)

タテハチョウ科マダラチョウ亜科に属するコモンマダラ属 *Tirumala* 全9種を対象にして, 雄の性標内で生成されるフェロモン運搬粒子 PTPs の微細構造の比較形態学的研究を行なった. 本属の種は, 外観だけでなく交尾器の形状も相互に酷似するため, 種間の識別が難しいとされていたが, 本研究において, PTPs の形態に明確な相違が見出され, 本属内の PTPs の微細構造は少なくとも4つのタイプに分けられることが明らかになった. さらに同所的に分布する種の PTPs の形態は異なるということが示唆された. 揮発性物質を運ぶ PTPs は, 同種の雌に交尾相手を正確に認識させる機能をもつものであると考えられる. つまり, *Tirumala* は種同士の外観が酷似するため, 特に他の種との生殖隔離機構が重要となる. モンシロチョウ属 *Pieris* において, 雄の発香物質の化学的成分やその成分比が種によって異なることがすでにわかっていることから, おそらく本属にもこのような種特異性があり, それとともにフェロモンを運ぶ PTPs の形態にも反映している可能性は十分ありうる.

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